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THE USE OF THE DOG-WHELK, *NUCELLA LAPILLUS*, AS AN INDICATOR OF TRIBUTYLTIN (TBT) CONTAMINATION

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(Figs. 1–10)

Two indices measuring the level of imposex induced by tributyltin (TBT) in populations of the dog-whelk, *Nucella lapillus*, are described. These are (i) the relative penis size, a measure of the mean size of the female penis in relation to that of the male; and (ii) the vas deferens sequence which recognizes six stages in the development of imposex, notably in the formation of the vas deferens. The use of these indices in the monitoring of populations is illustrated by data drawn from recent surveys of south-west England.

The evidence indicates that imposex, an apparently irreversible syndrome, is initiated by as little as 1 ng/l of tributyltin. Since *N. lapillus* is a long-lived species, surviving for six years or more, the level of imposex in adults can be considered to represent the response to long-term exposure to TBT contamination. However, imposex develops at an early age and its intensity in immature females, i.e. those about one year old, in the early stages of ovarian development and yet to breed for the first time, is thought to provide the most sensitive indicator of short-term contamination.

INTRODUCTION

The term 'imposex' was coined by Smith (1971) to describe the superimposition of male characters onto unparasitized and parasitized females of gonochoristic gastropods. In *Nassarius obsoletus* (Say) the development of imposex results in the female having one or more of the following characters: (1) a penis with a duct leading to (2) a vas deferens which passes back to the ventral channel of the capsule gland and (3) convolution of the normally straight gonadial oviduct (Smith, 1980). Imposex in the similarly gonochoristic *Nucella lapillus* (L.) was first noted by Blaber (1970) who found females with penis-like outgrowths in Plymouth Sound populations. Subsequent studies (Bryan *et al.* 1986; Gibbs & Bryan, 1986) have demonstrated that the incidence and intensity of imposex have since increased markedly in the same populations and that the phenomenon is widespread around south-west England.

There is now a substantial body of evidence indicating that imposex in stenoglossan gastropods, such as *N. obsoletus* and *N. lapillus*, is a recent syndrome arising from the introduction and increasing usage of antifouling coatings containing tributyltin (TBT) compounds, not only on boats but also on fish farm cages (see for example Davies, Bailey & Moore, 1987). In the case of *N. lapillus*, field observations show that close to harbours and marinas the reproductive capacity of populations is lowered to the point where few or no egg capsules or juveniles are present and a high proportion of the females exhibit advanced or

late imposex, that is, they contain aborted capsules as a result of blockage of the oviduct. Further, imposex, when measured by the relative size of the female penis, can be correlated with geographical position in relation to boating activity and also with tissue tin concentration. Experimentally, these relationships can be readily confirmed by transplanting *N. lapillus* from an area of low boating activity to a location close to a marina or harbour: usually within weeks the expression of imposex is significantly increased in the transplants. A similar increase in the degree of imposex can be induced by the direct application of TBT-containing paint to the shell spires of animals in the field or, in the laboratory, by exposing animals to the leachate of a paint containing TBT compounds. Overall, the evidence gained to date suggests that imposex in *N. lapillus* could be initiated by a concentration of tin, as tributyl species, of less than 1 ng/l. This high sensitivity of *N. lapillus* to TBT, the ease with which imposex can be recognised in the species and its wide geographic distribution make *N. lapillus* an ideal indicator or sentinel species for the detection of TBT contamination.

Smith (1980) measured the expression of imposex in *Nassarius obsoletus* by recognizing stages in the development of the penis and vas deferens and also in the degree of convolution of the gonadial oviduct. He concluded (Smith, 1981) that penis expression was the easiest character to evaluate and possibly this was the most sensitive indicator of contamination. Other workers, including Blaber (1970), Féral (1980) and Miller & Pondick (1984), found the percentage of penis-bearing females in *N. lapillus* populations to be a useful measure of imposex, but around south-west England at least, imposex has advanced to the stage where almost all females of this species are penis-bearing. However, experience has shown that penis size provides a reliable and convenient parameter of the degree of imposex in any *N. lapillus* population. Nevertheless, it gives no indication of the reproductive competency of a female that is afflicted with advanced or late imposex. Sterilisation of the female *N. lapillus* in the later stages of imposex is chiefly the result of vas deferens formation, not of penis development. As a means of gauging the reproductive status of a population, a sequence of six stages in the superimposition of male organs onto the female has been defined.

The present paper is intended as a guide to two methods which have been employed to assess the degree of imposex exhibited by *N. lapillus* populations; these are based on (1) the relative mean size of the penis in the two sexes (relative penis size (RPS) index), and (2) the sequence involved in the development of the vas deferens from early to late imposex (vas deferens sequence (VDS) index).

METHODS AND OBSERVATIONS

Recognition of the sexes and sex ratio

Sex determination of *N. lapillus* individuals in some populations can be difficult. Before the advent of widespread imposex, Feare (1970*a*) noted that the sex of living individuals can be determined with difficulty by the presence or

absence of a penis; criteria based on body colour and shell size prove unreliable. Clearly, for many present-day populations that are even only moderately affected by imposex, this distinction no longer holds, the difference in the penis size of males and females often being insufficient for reliable separation. The one

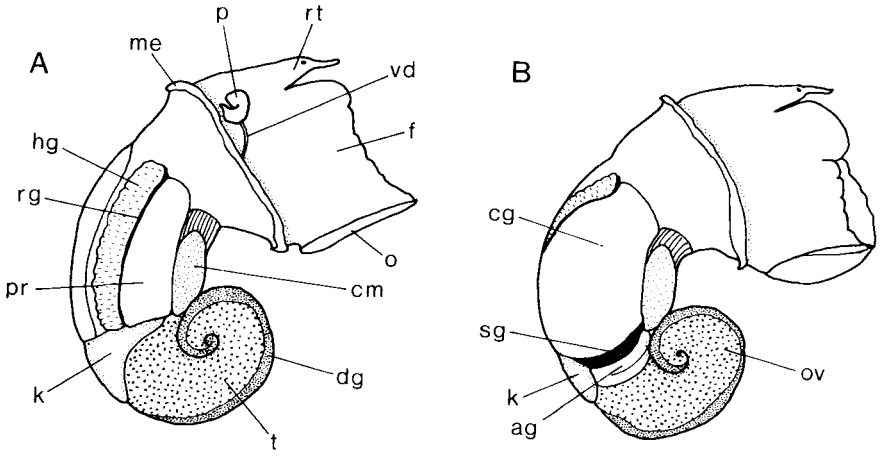

Fig. 1. *Nucella lapillus*. External features of mature male (A) and mature female (B) after shell removal. Abbreviations: ag, albumen gland; cg, capsule gland; cm, columella muscle; dg, digestive gland; f, foot; hg, hypobranchial gland; k, kidney; me, mantle edge; o, operculum; ov, ovary; p, penis; pr, prostate; rg, rectal gland; rt, right tentacle; sg, sperm-ingesting gland; t, testis.

character that appears to be distinctive and easily recognisable is the presence in the female of a sperm-ingesting gland, otherwise known as the 'brown gland' (see Pierson, 1955). As the latter name implies, this gland is brownish in colour, but often has a reddish tinge, and it is situated immediately posterior to the capsule gland (Fig. 1). Unlike the gonadial tissue, the size and colour of the sperm-ingesting gland does not appear to vary with the reproductive state of the female. Males with tissue having the appearance of a sperm-ingesting gland have been found, but such aberrant individuals appear to occur very rarely, at least in south-west England populations. Overall the presence of a sperm-ingesting gland provides the most convenient external body character for the recognition of a female.

Females often predominate in populations of gonochoristic molluscs and, probably because of differential mortality, this inequality often becomes accentuated with age. In *N. lapillus* populations both Pelseneer (1926) and Feare (1970*a*) concluded that the proportion of females increases with age. However, in populations severely affected by imposex, frequently this trend is reversed, males outnumbering females (Bryan *et al.* 1986); higher female mortality may result from the blockage of the pallial oviduct and the subsequent accumulation of aborted capsules (Gibbs & Bryan, 1986).

The sex ratios obtained in samples of a single *N. lapillus* population often show wide variation; for example in the 28 monthly samples of 100 individuals taken

from a North Sea population, Feare (1970*a*) found the proportion of females varied between 31 and 64%. Similarly in 12 samples of 20–31 individuals taken over a 16 month period at Widemouth Bay (Fig. 3), females comprised between 38 and 75% of the samples. *N. lapillus* is known to exhibit aggregative behaviour during certain months (see Feare, 1971); clearly this behaviour contributes towards sampling variability and some allowance for it has to be made in interpreting any sex ratio that shows a significant departure from equality.

Relative penis size (RPS) index

The relative penis size (RPS) index for any population is defined as the mean bulk of the female penis expressed as a percentage of the mean bulk of the male penis. The bulk of a penis is calculated as the cube of its length.

Measurement of the length of a penis in *N. lapillus* is a simple procedure since the penis can be exposed without dissection by placing the animal (minus shell) dorsal side up and drawing back the flap of tissue forming the roof of the mantle cavity. Under a binocular microscope and using 1 mm-graduated graph paper the length of the penis can be measured to the nearest 0·1 mm from its tip to its base, i.e. junction with the body wall behind the right tentacle. No attempt is made to straighten the penis which usually presents itself as a relatively immobile, stout structure. Bifurcate or even trifurcate penes are occasionally found, particularly on females, and in these cases the longer or longest structure is measured. A female lacking any measurable penial outgrowth is registered as zero and this value included in the calculation of the mean.

In *N. lapillus* males, penis size is related to body size, the latter being generally subject to considerable variation between populations according to environment. To quote extreme examples, the exposed population at Cape Cornwall (near Land's End: grid ref. SW351317) is composed of generally small-sized individuals and the mean male penis length is only $3{\cdot}08 \pm 0{\cdot}29$ mm ($n = 13$) whilst that of the much larger individuals in the more sheltered population at Torcross (East Devon: SX824416) is 50% greater at $4{\cdot}58 \pm 0{\cdot}54$ mm ($n = 39$). The effect of such variability is reduced by calculating the RPS value based on a comparison of female and male penes sizes in individuals from the same population.

Whilst length is the most convenient parameter of penis size to measure, it does not convey a true impression of the difference in mass between, say, a penis of 1 mm length and one of 3 mm. However, the weight (or volume) of the penis is related to the cube of its length (Bryan *et al.* 1986, fig. 2) and thus RPS values can be calculated by: (mean length of female penis^3/mean length of male penis^3) $\times$ 100. An RPS value of 50% indicates that the mean penis size of the female is half the bulk of that of the male.

RPS values give a reliable measure of imposex development in all areas except those where contamination is severe. In these areas the male penis is often deformed, its smooth outline being obscured by nodular excrescences which usually increase the measurable length; consequently, the RPS value is lowered and imposex thereby underestimated.

If sampling is restricted to mature adults, i.e. those with a thickened shell lip and usually with teeth, characters signifying the cessation of growth (Crothers, 1985), then the observed variation in penis length in both males and females in a population is generally quite small. This feature is illustrated by the data for

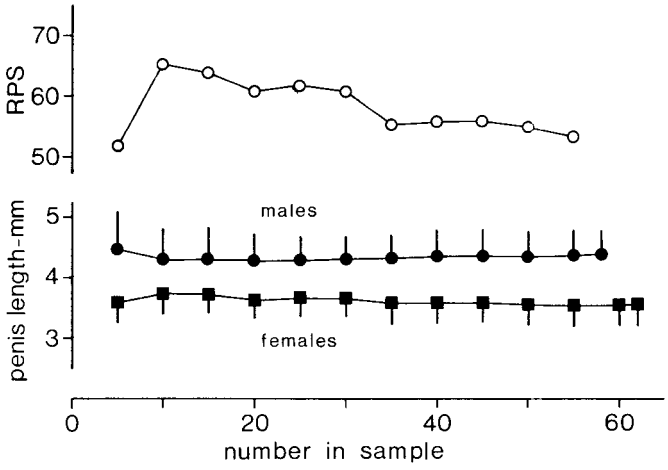

Fig. 2. *Nucella lapillus*. Effect of sample size on estimates of mean penes lengths and relative penis size (RPS) index. Based on a sample of 58 males and 62 females from Tregantle, west of Plymouth (SX391523) taken 25 October 1986. Values shown are means (standard deviations indicated by vertical lines) for 5, 10, 15, ..., animals of each sex calculated cumulatively.

the Tregantle population shown in Fig. 2. The same data can be used to gain some idea of the effect of sample size on RPS values: the total sample of this population comprised 120 individuals (58 males, 62 females) and this number gave a RPS value of 53%; if a smaller sample of between, for example, 10–40, equal sexes, had been taken an RPS value between 51 and 65% would have resulted, also indicating well-developed imposex; thus large samples are not essential to obtain good RPS estimates. Other data, involving a less affected population repeatedly sampled over many months, also indicate that small samples give reproducible RPS values. The seasonal analysis of the Widemouth population (Fig. 3) is based on samples of 20–31 animals with male:female ratios varying from 5:15 to 18:11; nevertheless, the variation in the RPS is slight, i.e. 0·6–2·9%.

Consistent RPS values can be obtained from analyses by different workers. The data obtained by two workers independently measuring the penes of the same animals in samples from two populations are given in Table 1. Despite the small sample sizes and different imposex levels the RPS values show good agreement for the separate analyses. Other interlaboratory analyses of 'test' populations have yielded RPS values varying by only 1 or 2%.

The use of the RPS index of *N. lapillus* in assessing the geographical distribution of imposex and its intensity around coasts is illustrated by the results of a survey of west Cornwall and the Isles of Scilly (Fig. 4). Away from centres of boating activity, RPS values are low, 5% or less, but increase sharply to 40%

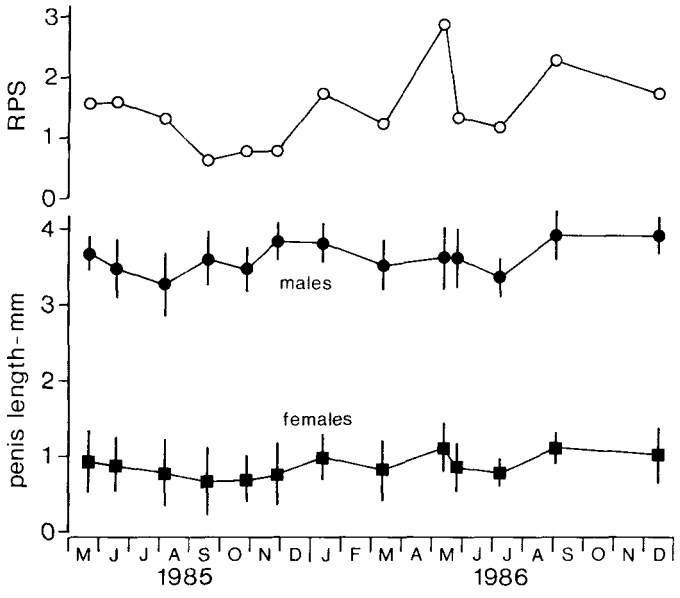

Fig. 3. *Nucella lapillus*. Effect of season on penis length of males and females and on RPS index. Values are means (standard deviations indicated by vertical lines) for samples of 20–31 animals from Widemouth Bay.

Table 1. Nucella lapillus. *Comparison of data obtained by two workers, A and B, independently measuring penis lengths of same animals in two populations having different imposex levels (samples collected 25–26 Oct. 1986)*

	Penis length (mm)				
	Male		Female		RPS
	Mean ± S.D.	*n*	Mean ± S.D.	*n*	(%)
	Trebarwith Strand (SX048865)				
A	4·25 ± 0·344	14	1·44 ± 0·696	14	3·9
B	4·20 ± 0·368		1·49 ± 0·698		4·5
	Tregantle (SX391523)				
A	4·33 ± 0·500	14	3·69 ± 0·315	16	62·1
B	4·20 ± 0·457		3·62 ± 0·383		64·0

or more near the harbours of Porthleven, Penzance, St Ives and Hugh Town (St Mary's). Such RPS values reflect the extent of contamination by TBT compounds emanating from antifouling paints.

Vas Deferens Sequence (VDS) index

The size of the female penis relative to that of the male is a convenient measure of the intensity of imposex in *N. lapillus* and of TBT contamination but, as explained above, gives no indication of reproductive competency. In this respect the formation of the vas deferens is more significant since not only does the development of this structure precede that of the penis but also it eventually

causes the pallial oviduct to be occluded. The recognition of different stages in vas deferens development therefore provides a more sensitive method of categorizing the intensity or expression of imposex. The vas deferens is fully displayed if the tissue forming the mantle cavity roof is cut longitudinally close

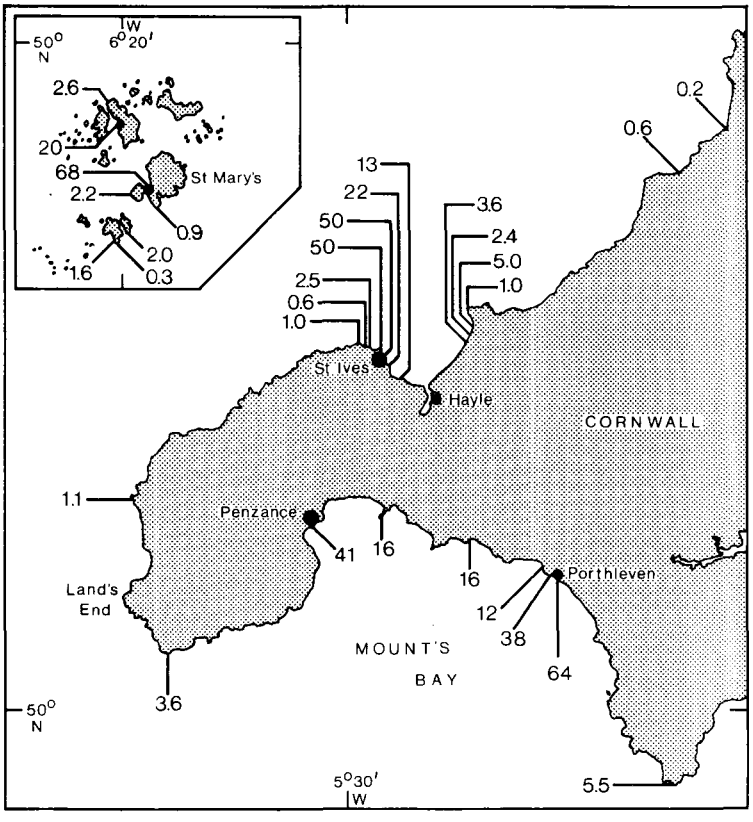

Fig. 4. *Nucella lapillus*. Relative penis size (RPS) indices for sites and populations around west Cornwall and Isles of Scilly based on samples taken during 1984–6.

to the hypobranchial gland and the capsule gland then rotated to expose its medial surface (Gibbs & Bryan, 1986, fig. 1 B).

The vas deferens is a duct formed by infolding of the mantle cavity epithelium and subsequent fusion of the edges, the line of closure remaining traceable throughout the length of the duct (Fretter & Graham, 1962). In some species, for example *Nassarius obsoletus*, the vas deferens apparently develops progressively from the base of the penis to the pallial oviduct (see Smith, 1981) but in *N. lapillus* the female vas deferens develops from two centres. Initially, there is an infolding of the epithelium close to the ventral margin of the prominent genital papilla surrounding the female opening or vulva; from this centre the infolding progresses in a medio-anteriad direction to form the proximal section of the vas deferens. As penis formation commences, the distal section develops from the

posterior base of the penis to meet and fuse with the proximal section, typically at a point about one-third of the distance between the penis and genital papilla; the length of the distal section is thus about half that of the proximal.

Females with a well-developed penis and complete vas deferens appear to be

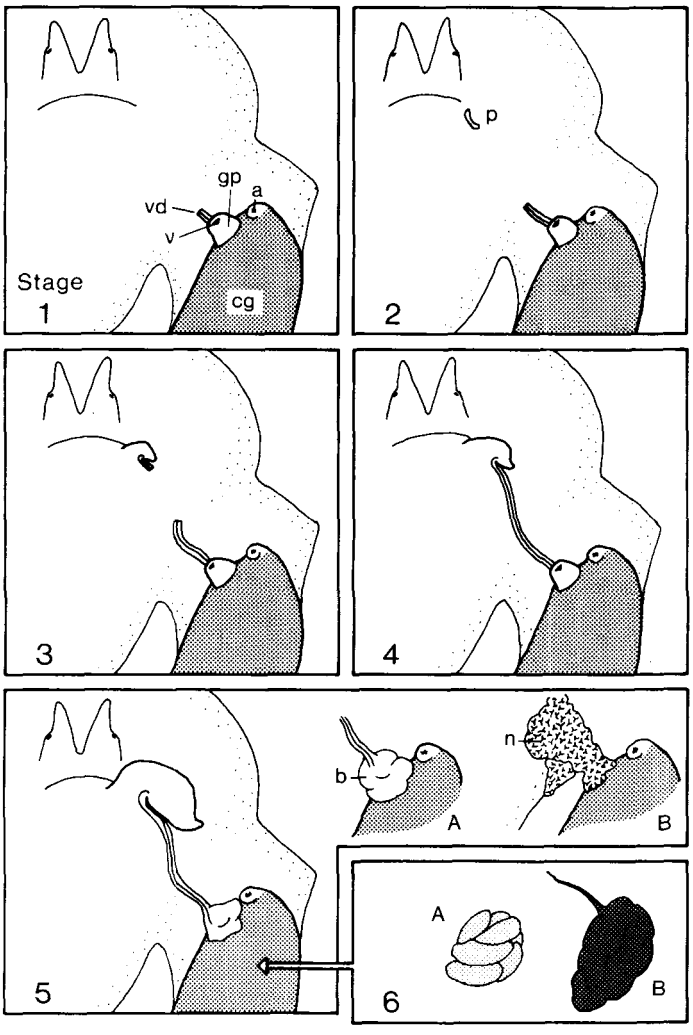

Fig. 5. *Nucella lapillus*. Stages in the development of imposex based on vas deferens sequence (VDS). See text for explanation. Abbreviations: a, anus; b, 'blister'; gp, genital papilla; n, 'nodule'; p, penis; v, vulva; vd, vas deferens.

capable of breeding provided the opening (vulva) of the pallial oviduct remains unobstructed. However, in areas close to harbours and marinas, vas deferens tissue continues to be proliferated ('hyperplasia') and at the duct's proximal end this tissue eventually obscures the genital papilla and occludes the vagina, effectively preventing the expulsion of capsules. Capsules thus aborted accumu-

late in the capsule gland, in some cases to the extent that this organ ruptures. Although the presence of large capsule masses within the capsule gland is usually manifested externally because of distension of the gland wall (see Gibbs & Bryan, 1986, plate I) a longitudinal incision of the gland is necessary routinely to ascertain the presence or absence of any aborted capsules within the lumen.

The vas deferens sequence (VDS) in imposex development may be categorized from Stage 0 (normal) to Stage 6 (grossly affected). The stages are illustrated in Fig. 5 and can be defined as follows.

Stage 0 – the 'normal' female state with no male character being visibly superimposed; pallial oviduct terminates at a clearly defined opening or vulva situated at the apex of a prominent genital papilla which projects into the mantle cavity.

Stage 1 – development of proximal section of vas deferens commencing by infolding of the mantle cavity epithelium in the region ventral to the genital papilla.

Stage 2 – development of penis initiated with the formation of a ridge (usually sigmoid) a short distance behind the right tentacle.

Stage 3 – small penis formed and development of distal section of vas deferens commencing from base of the penis.

Stage 4 – proximal and distal sections of vas deferens now fused and penis enlarging to a size approaching that of the male.

Stage 5 – proliferating vas deferens tissue overgrowing genital papilla causing vulva to be displaced, constricted or no longer visible; blister-like protuberances may appear around site of the papilla (Fig. 5:5A) and 'nodules' of hyperplasic tissue often develop (Fig. 5:5B).

Stage 6 – lumen of capsule gland contains the material of aborted capsules; this material may comprise a single capsule or several to many that are compressed together to form a translucent or light to dark brown mass (Fig. 5:6A, B).

The sequence of events leading to the sterilization of the female *N. lapillus* is described more fully in Gibbs & Bryan (1986). In that paper the VDS was abbreviated to three stages, namely, 'early', 'intermediate' and 'late'; 'early' imposex corresponds to VDS stages 1, 2 and 3, 'intermediate' to stage 4 and 'late' to stages 5 and 6. Comparison of different populations showed that there was a general trend in the prevalence of early, intermediate and late stages that corresponded to the distance from a source of TBT contamination, with early stages dominating in populations living in 'clean' areas and late stages dominating in populations close to marinas or harbours. However, recognition of six stages rather than three provides a more critical method for the appraisal of the level of imposex in any population.

The use of the vas deferens sequence is illustrated in Fig. 6 which compares the intensity of imposex in adult female *N. lapillus* in populations at nine sites around south-west England – five along the relatively uncontaminated north coast and four in the heavily contaminated areas of Plymouth Sound and Tor Bay. These data demonstrate the prevalence of imposex around the south-west

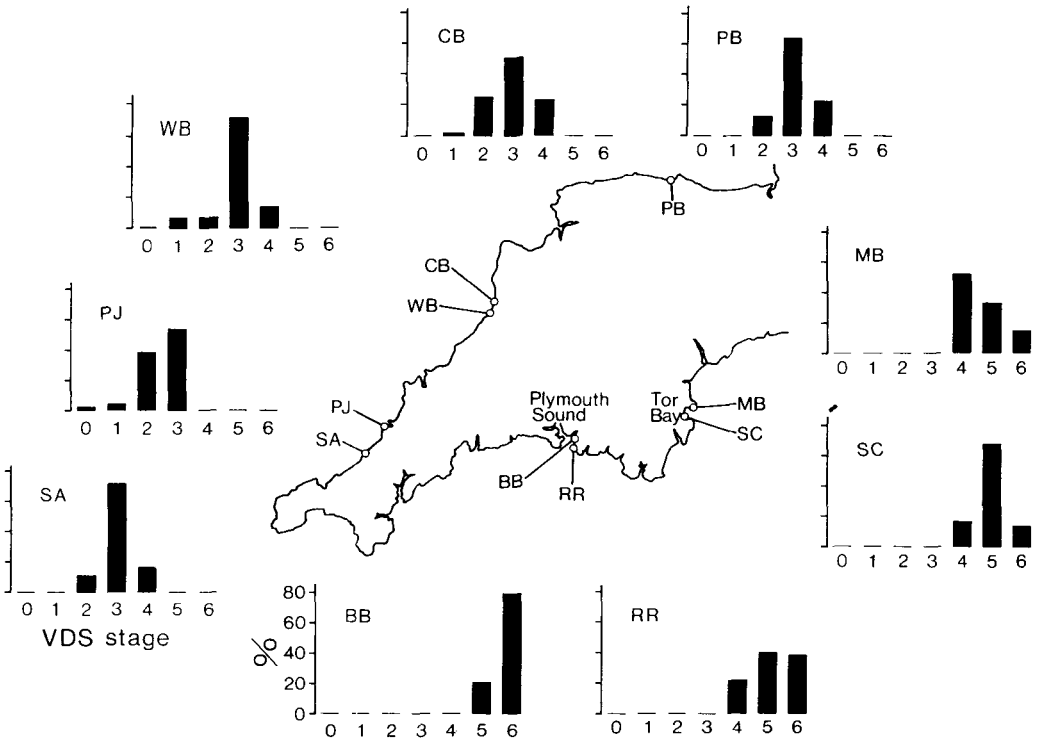

Fig. 6. *Nucella lapillus*. Imposex levels in selected populations around south-west England based on the vas deferens sequence (VDS). See Table 2 for details.

Table 2. Nucella lapillus. *Data relating to the RPS and VDS analyses of imposex development in the nine populations shown in Fig. 6*

Site	Grid ref.	Sampling date (1986)	Females in sample		RPS	VDS index	Concentration of hexane-extractable tin (μg/g dry tissue)*	
			n	%	(%)		TBT fraction	Total (TBT+DBT)
St Agnes (SA)	SW722518	4 Nov.	61	55·5	0·55	3·05	0·113	0·129
Porth Joke (PJ)	SW768607	25 Feb.	67	54·5	0·003	2·43	0·047	0·060
Widemouth Bay (WB)	SS194015	25 May	71	54·2	1·31	2·89	0·040	0·105
Crooklets Beach (CB)	SS201072	28 Mar.	89	58·7	2·00	2·93	0·087	0·108
Porlock Bay (PB)	SS861487	8 July	56	35·7	2·15	3·10	0·036	0·085
Meadfoot Beach (MB)	SX931631	9 June	60	31·4	30·6	4·63	0·132	0·271
Saltern Cove (SC)	SX895586	23 June	65	33·3	46·5	4·95	0·136	0·298
Renney Rocks (RR)	SX491487	10 Apr.	55	38·9	26·5	5·16	0·178	0·351
Batten Bay (BB)	SX487530	10 Feb.	34	22·9	53·2	5·79	0·633	0·963

* Whole body concentrations of 6 unstaged females (pooled): see Bryan *et al.* 1986 for method of determination.

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peninsula: even the north coast populations exhibited moderately developed imposex (53–88% at stage 3 or 4) and surprisingly few (3 out of 344 females) totally lacked any outward sign of imposex. However, no female from this coast had reached stage 5 and evidently the reproductive capacity of these populations remains unimpaired. This is in contrast to the state of the Plymouth Sound and Tor Bay populations in which all females examined ($n = 214$) had progressed beyond stage 3 and a high proportion of each population was assessed to be sterile having reached stages 5 or 6; in the case of the Batten Bay population, no reproductively active female was found.

Calculation of the mean VDS stage provides an index by which to compare imposex in different populations. Any population with an index above 4 contains sterilised females and thus has a reduced reproductive capacity. In the case of the north coast populations VDS indices vary between 2·4 and 3·1 whilst those for the south coast are much higher, between 4·6 and 5·8 (Table 2). The concentrations of hexane-extractable tin in the tissues of the females (Table 2) likewise reflect the degree of contamination of the two coastlines.

Long-term monitoring

RPS and VDS analyses provide methods of quantifying long-term changes in the intensity of imposex affecting *N. lapillus* populations. Up to the present, the only evidence for long-term change has been restricted to a single example, i.e. the re-survey of the Plymouth Sound populations studied by Blaber in 1969 (see Bryan *et al.* 1986). Whilst the intensity of imposex can be demonstrated experimentally to increase markedly with increasing TBT contamination, for example by transplanting animals from a 'clean' site to one close to a marina, a demonstration of the reverse process does not appear to be possible since all of the evidence points to imposex being an irreversible syndrome. Following any governmental legislation to restrict or ban the use of TBT as an antifouling agent the problem of detecting any *decrease* in the intensity of imposex becomes imperative in respect of monitoring changes in environmental levels.

In the surveys carried out to date, mature adults have been used rather than immature individuals because they are more easily sexed, being larger they offer more tissue for chemical analyses and also in severely contaminated areas they are the only animals available. However, imposex develops at an early age, often to the extent that it is not possible to sex juveniles on the basis of their external characters until the sperm-ingesting gland can be distinguished: in most south-west England populations this occurs when the juveniles have reached 10–15 mm length and are probably 6–12 months of age. Even when this young, females that are identifiable as such, may exhibit imposex up to stage 4 and possibly stage 5. On this evidence it may be supposed that the extent of imposex depends on the level of contamination from the time of hatching or even earlier.

The effect of different TBT concentrations on the development of imposex in juvenile *N. lapillus* has been investigated experimentally. Batches of egg capsules, collected from the 'clean' site at Crooklets Beach in October 1985, were placed

in two tidal tanks ('control' and experimental) both supplied with laboratory circulation water pumped from Plymouth Sound. TBT levels in this water supply fluctuate with seasonal boating activity, but for the year 1986 a mean concentration of *ca.* 1·5 ng/l TBT tin was determined for the 'control' tank water (see

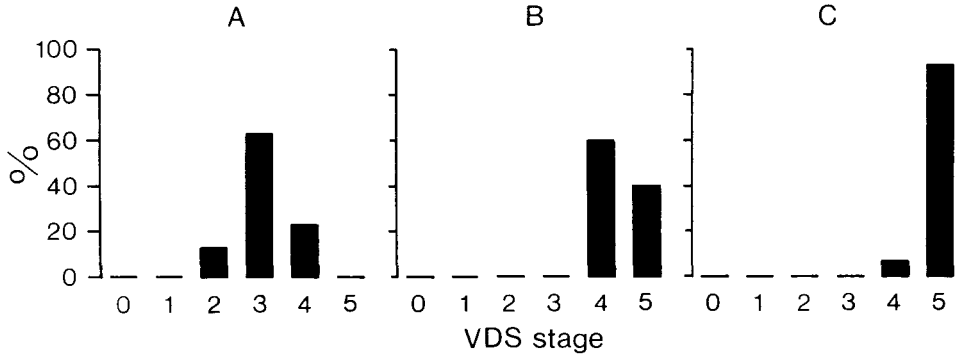

Fig. 7. *Nucella lapillus*. Vas deferens sequence (VDS) stages in (A) field, (B) 'control' and (C) experimental populations of immature (18–22 mm length) females after *ca.* 12 months exposure to < 0·5, 1·5 and 18·7 ng/l of tin (TBT fraction) respectively. See text for explanation and Table 3 for other details.

Table 3. Nucella lapillus. *Comparison of imposex development in immature individuals (18–22 mm in length) after one year of exposure to different concentrations of tributyltin*

Data for adults (24–29 mm) from stock population at Crooklets (collected Nov. '86) are also given. See also Fig. 7.

Population (TBT tin level)		Number in sample	Mean wet wt (g)	Penis length (mm) $\bar{x} \pm$ S.D.	RPS (%)	VDS index
Field	♂	34	0·365	2·03 ± 0·53	5·15	3·10
(< 0·5 ng/l)	♀	30	0·346	0·76 ± 0·39		
'Control'	♂	10	0·585	2·74 ± 2·27	63·9	4·40
(1·5 ng/l)	♀	10	0·575	2·36 ± 0·32		
Experimental	♂	12	0·523	3·05 ± 0·18	95·8	4·94
(18·7 ng/l)	♀	14	0·571	3·01 ± 0·19		
Field adults	♂	8	0·95	3·99 ± 0·24	2·52	2·93*
(< 0·5 ng/l)	♀	12	1·11	1·17 ± 0·73		

* Based on data for 28 March 1986 (Table 2).

Bryan *et al.* (1987) for method of determination). The TBT level in the water supplied to the experimental tank was enhanced using the leachate of a copolymer antifouling paint (International, 'Cruiser') to a mean tin level of 18·7 ng/l. In both tanks, control and experimental, juveniles hatched during December 1985 and, maintained on a clean diet of barnacles and mussels, many attained a shell length of 18–22 mm after 12 months. In December 1986, samples from both tanks

were examined for imposex along with a sample of similar-sized animals collected from the same 'stock' population at Crooklets, a site where the TBT tin level is very low – < 0·5 ng/l.

The results of this experiment are given in Fig. 7 and Table 3. These show that penis size in both male and female increases with exposure to increasing TBT levels, to the extent that at about 20 ng/l of tin (TBT fraction) there is little difference between the sexes (RPS = 96%). In terms of the VDS the stages exhibited by the immature Crooklets individuals correspond to those of the adult population (shown in Fig. 6) with stage 3 predominating; however, the VDS is advanced considerably to include only stages 4 and 5 when the TBT level is raised. Significantly, at around 20 ng/l of tin (TBT fraction) nearly all immature females were assessed to have blocked oviducts and thus had been rendered sterile before reaching their first breeding season. This conclusion was supported by light microscopy sections of 3 examples, each of which showed the vas deferens tissue to have invaded the anterior region of the pallial oviduct causing its occlusion.

These observations indicate that imposex in immature females, rather than that in females that have reached maturity, provides a more sensitive indicator of short-term changes in contamination levels. Routine monitoring of the RPS and VDS on a yearly basis of the immature cohort of a population would provide a quantitative assessment of any deterioration or improvement in ambient water quality.

DISCUSSION

Nucella lapillus has a unique combination of characters that make it a prime candidate for use as an indicator of TBT contamination. Briefly, (1) it has a wide geographical distribution on both sides of the North Atlantic; (2) where found it is usually common, conspicuous and easily indentified (see Fretter & Graham, 1985); (3) it has very limited potential for dispersal since its development is direct and the adults are slow-moving, remaining in the same general shore area throughout life; (4) its eggs are laid in a conspicuous capsule that survives many months thus providing a convenient marker of breeding activity; (5) the species is hardy, allowing for experimentation, such as transplantation from one area to another and (6) most importantly, its sensitivity to TBT, as manifested by imposex, is seemingly unrivalled.

Imposex in *N. lapillus* is a readily observed phenomenon that follows a defined sequence of events and thus lends itself to comparative analysis either by the quick method of relative penis size calculation or the more time-consuming method of VDS stages. The correlations between RPS and VDS indices and the concentrations of tin, as tributyl species, in ambient sea water and in the tissues of the female are shown in Figs. 8 and 9. The data demonstrate the high sensitivity of *N. lapillus* to TBT in that the intensity of imposex, as expressed by penis and vas deferens formation, increases rapidly at remarkably low concentrations, both in the water and in the female tissues. The correlation between the proportion

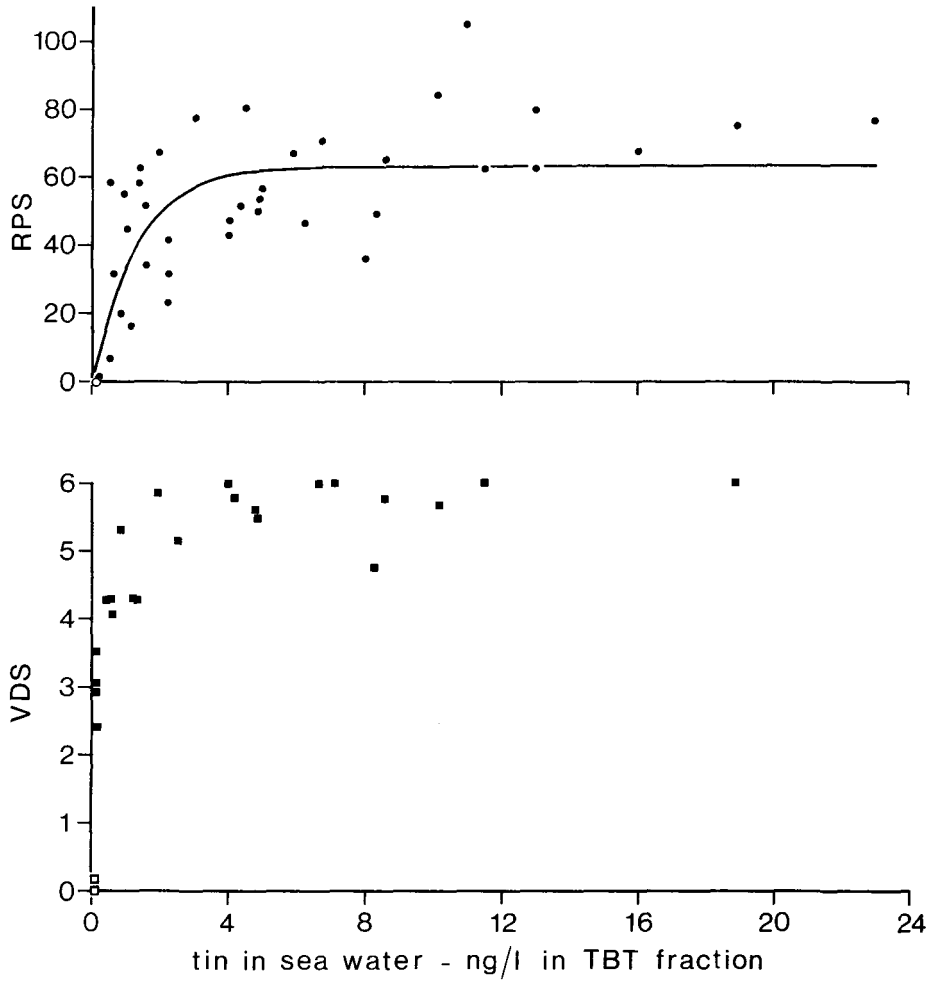

Fig. 8. *Nucella lapillus*. Relationship between (A) relative penis size (RPS) and (B) vas deferens sequence (VDS) indices and concentrations of TBT tin in ambient sea water for samples of animals and water taken at the same time around south-west England, 1984–86. (Values for two Isle of Mull samples (open symbols) are also included.) Equation of line: RPS = $75(1-e^{-0\cdot408Sn})$. ($n = 39$.)

of sterile females (i.e. those at stages 5 and 6) found in populations and ambient tin concentrations (TBT fraction) around south-west England is summarized in Fig. 10. Some sterile females appear in populations exposed to levels as low as 1 or 2 ng/l of tin; at 4 ng/l about half of the females appear sterilised and above this level reproductive activity becomes severely curtailed. In places where the TBT tin concentration reaches 7–10 ng/l no breeding appears to occur.

There is no evidence that TBT-induced imposex is fatal in the short-term, nor that it is reversible. Thus imposex in mature adults, some of which survive for six years (Feare, 1970*b*) but possibly live for a decade or more, reflects long-term exposure to TBT. Short-term exposure levels can be best gauged by the degree

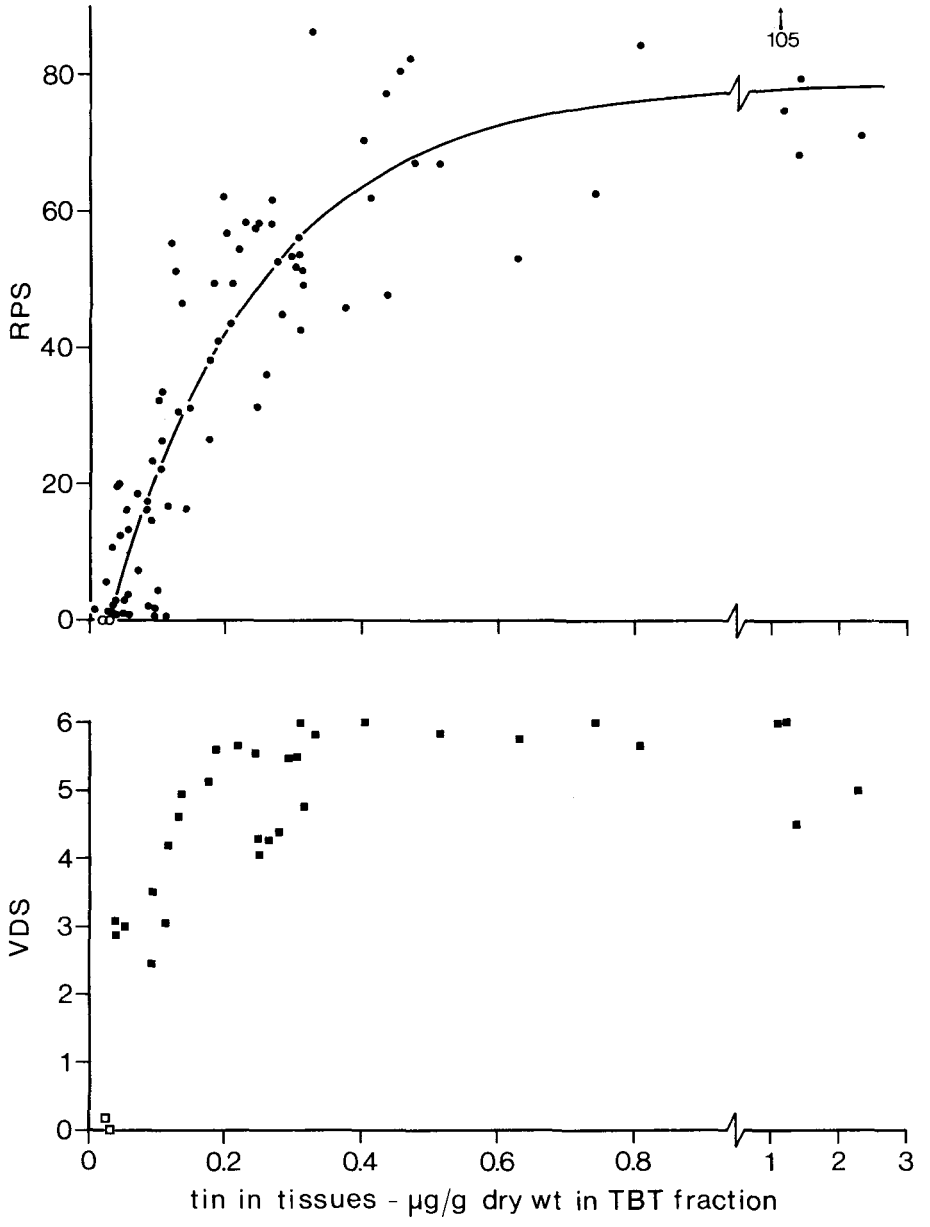

Fig. 9. *Nucella lapillus*. Relationship between (A) relative penis size (RPS) and (B) vas deferens sequence (VDS) indices and whole body concentrations of TBT tin in females from south-west England populations 1984–86. (Values for two Isle of Mull samples (open symbols) also included.) Equation of line: $RPS = 89{\cdot}5(1-e^{-4{\cdot}446Sn})-10{\cdot}9$. ($n = 90$). Note change in scale.

of imposex in immature females that have yet to breed; individuals 12–18 months old seem to have the greatest potential for monitoring purposes.

Much of the present study has centred on *N. lapillus* around south-west England. It should be added that the imposex appears to be widespread in

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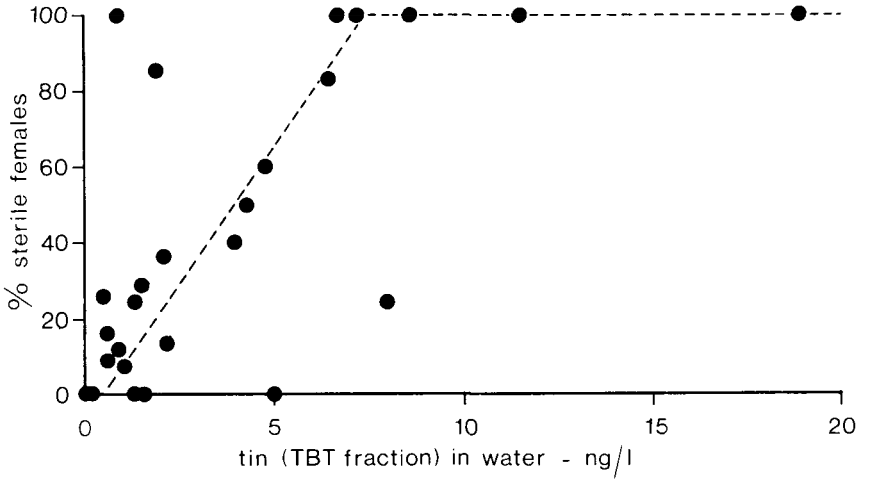

Fig. 10. *Nucella lapillus*. Relationship between the concentration of hexane-extractable tin (TBT fraction) in ambient sea water and percentage of sterile females (VDS stages 5 and 6) in south-west England populations. Broken line is eye-fitted.

N. lapillus throughout its range. In British waters the syndrome has been found at all localities where it has been searched for (except at one site on the Isle of Mull, in the Western Isles of Scotland) and it has been found in samples from France (Roscoff: see also Féral, 1980) and N.W. Spain (Noya, Galicia). Further afield it is recorded in New England populations (Miller & Pondick, 1984).

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REFERENCES

BLABER, S. J. M., 1970. The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* (L.). *Proceedings of the Malacological Society of London*, **39**, 231–233.

BRYAN, G. W., GIBBS, P. E., BURT, G. R. & HUMMERSTONE, L. G., 1987. The effects of tributyltin accumulation on adult dog-whelks, *Nucella lapillus*: long-term field and laboratory experiments. *Journal of the Marine Biological Association of the United Kingdom*, **67**, 525–544.

BRYAN, G. W., GIBBS, P. E., HUMMERSTONE, L. G. & BURT, G. R., 1986. The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, **66**, 611–640.

CROTHERS, J. H., 1985. Dog-whelks: an introduction to the biology of *Nucella lapillus* (L.). *Field Studies*, **6**, 291–360.

DAVIES, I. M., BAILEY, S. K. & MOORE, D. C., 1987. Tributyltin in Scottish sea lochs, as indicated by degree of imposex in the dogwhelk, *Nucella lapillus* (L.). *Marine Pollution Bulletin*, in press.

FEARE, C. J., 1970*a*. The reproductive cycle of the dog-whelk (*Nucella lapillus*). *Proceedings of the Malacological Society of London* **39**, 125–137.

FEARE, C. J., 1970*b*. Aspects of the ecology of an exposed shore population of dogwhelks *Nucella lapillus* (L.). *Oecologia*, **5**, 1–18.

FEARE, C. J., 1971. The adaptive significance of aggregation behaviour in the dogwhelk, *Nucella lapillus* (L.). *Oecologia*, **7**, 117–126.

FÉRAL, C., 1980. Variations dans l'évolution du tractus génital mâle externe des femelles de trois gastéropodes prosobranches gonochoriques de stations Atlantiques. *Cahiers de biologie marine*, **21**, 479–491.

FRETTER, V. & GRAHAM, A., 1962. *British Prosobranch Molluscs*. London: Ray Society.

FRETTER, V. & GRAHAM, A., 1985. The prosobranch molluscs of Britain and Denmark. Part 8. Neogastropoda. *Journal of Molluscan Studies*, supplement **15**, 435–556.

GIBBS, P. E. & BRYAN, G. W., 1986. Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, **66**, 767–777.

MILLER, E. R. & PONDICK, J. S., 1984. Heavy metal levels in *Nucella lapillus* (Gastropoda: Prosobranchia) from sites with normal and penis-bearing females from New England. *Bulletin of Environmental Contamination and Toxicology*, **33**, 612–620.

PELSENEER, P., 1926. La proportion rélative des sexes chez lex animaux et particulièrement chez les mollusques. *Mémoires de l'Académie Royale de Belgiques* (*Classe des sciences*), **8**, 1–258.

PIERSON, M., 1955. Particularités histologiques de la glande brune chez *Nucella lapillus* (L.). (Gastéropode prosobranche). *Compte rendu hebdomadaire des séances de l'Académie des sciences*, **241**, 1168–1170.

SMITH, B. S., 1971. Sexuality in the American mud-snail, *Nassarius obsoletus* Say. *Proceedings of the Malacological Society of London*, **39**, 377–378.

SMITH, B. S., 1980. The estuarine mud snail, *Nassarius obsoletus*; abnormalities in the reproductive system. *Journal of Molluscan Studies*, **46**, 247–256.

SMITH, B. S., 1981. Male characteristics on female mud snails caused by antifouling bottom paints. *Journal of Applied Toxicology*, **1**, 22–25.